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The Effects of Various Cation Concentrations on Salivary Amylase Activity

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THE EFFECTS OF VARIOUS CATION CONCENTRATIONS ON SALIVARY AMYLASE ACTIVITY

BY

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LIFE

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CHAPTER I

INTRODUCTION AND STATEMENT OF PROBLEM

Present in both saliva and pancreatic juice is a starch-splitting enzyme, alpha-amylase, (α-1, 4-glucan 4 glucanohydralase - E.C. 3.2.1.1) a protein. This enzyme acts on α, 1, 4 glucoside links, catalyzing the hydrolysis of starch (or glycogen) through a number of carbohydrate products of progressively shorter chain lengths, called dextrins, until maltose is formed. Further hydrolysis of maltose to glucose does not occur to any significant degree in saliva even though slight traces of maltase are present.

The optimum pH for salivary amylase is 6.8 and optimum temperature is 40^{o} C. One can see that the oral cavity is an ideal environment since the pH is 6.5-7.3 and the temperature is 37^{o} C.[24]

The optimum pH and temperature of pancreatic amylase is 6.5-7.2 and 37-40^{o} C.[7]

Food is actually swallowed before the salivary amylase can react with it to a great degree. The hydrolysis of starch is taken over and completed by the pancreatic amylase. The salivary enzyme's important function is to initiate the digestive process.[24]

The salivary concentration of potassium is relatively high, and that of sodium is relatively low, when compared to that normally found in extracellular fluids. The pancreatic secretion has a sodium-potassium content dissimilar to that of saliva.

Knowing the few facts related above several questions come to mind: Does the difference in sodium and potassium concentration of the two body fluids appreciably affect the amylolytic activity of the salivary and pancreatic amylase respectively? Does the presence of two cations enhance or decrease the activity of the two amylases? Do the amylases react in a similar manner? Do other cations have any effect on activity?

With these questions in mind, it was decided to determine the effects of sodium and potassium concentration on the enzymic activity of salivary and pancreatic alpha amylase. The effect of calcium and magnesium was also investigated.

The cation concentration was varied considerably, but all other conditions remained as constant as possible. All times and temperatures remained constant. All volumes, and concentrations of the substrate and enzyme also remained constant.

CHAPTER II

REVIEW OF THE LITERATURE

Digestion is the process of breaking down molecules of carbohydrates, fats, and proteins. The majority of compounds which one ingests are composed of single molecules joined together by bonds formed with the removal of water.[24]

The digestive reactions are hydrolysis reactions catalyzed by specific enzymes secreted in various parts of the gastrointestinal tract. These enzymes enter the tract in the different digestive juices: saliva, gastric, pancreatic, and intestinal.[24] These reactions occur in living cells because of natural catalysts, called enzymes. These enzymes lower the energies required in the reaction sufficiently for the reactions to occur under the milder condition of the human body.[22] All the known enzymes are proteins.[22] The enzymes not only speed up the reaction but also direct the reaction to specific pathways and thus are part of cellular organization mechanisms.[22]

The enzyme action is facilitated by the folding of the protein chain to provide one or more active areas which specifically fit a substrate molecule and hold it briefly.[23]

Human saliva contains an enzyme, alpha-amylase, capable of digesting starch or glycogen.[20] There is approximately

0.4 gram/liter of amylase in human saliva which is about 12% of the total amount of organic material.[20] Some sources say in addition to alpha-amylase being present in human saliva that there is also a beta-amylase,[12] but the prevailing view says that beta-amylase, is not present in humans,[19] or in animal sources in general.[3] A salivary sample containing amylase has been shown to have 1/22 of the activity of that of commercial amylase.[17]

Most of the amylase in human saliva is from the parotid gland.[16] In regards to amount, man is second only to the rat in salivary amylase content.[18]

The activity of salivary amylase seems to be extremely variable. It varies during the day, and depends on meals and many other variants.[40] The activity can even vary on different days in the same individual. The highest values appearing before meals.[36] Another source, however, states that activity is highest when the enzyme is collected after eating.[2] Smoking, toothpaste, and water decrease the activity of salivary amylase.[36]

The optimum pH for salivary amylase activity is 6.6, the optimum temperature is 40^{o} C. The activity is inhibited below pH 4.0.[24] With these figures in mind one can see that the oral cavity is an excellent environment for amylase

activity in that it has a pH of 6.5-7.3 and a temperature of 37° C.

In vitro, salivary alpha-amylase acts on the ∝ -1, 4 glucoside links, catalyzing the hydrolysis of starch (or glycogen) through a number of carbohydrate products of progressively shorter chain lengths, called dextrins, until maltose is formed.[24] Further hydrolysis of maltose to glucose does not occur, even though traces of maltase are present in saliva.[24] In vivo, food is actually swallowed before saliva can react with it to a significant degree. It is important, however, in that it initiates digestion.[24]

Hydrolysis of starch by salivary amylase yields 73% maltose and 15% "non-fermentable" dextrins. No correlation between inhibition of the enzyme by maltose or glucose and the formation of dextrins can be seen.[31]

The presence of maltose increases the quantity of salivary amylase production but decreases the action of the enzyme. Lactose decreases production and increases activity. Glucose and fructose decrease activity.[39]

Saliva contains 3.0-12.0 meq./liter of sodium and 7.5-9.5 meq./liter of potassium[26] as determined by Chally and Foulk method. Its chloride content varies greatly: 40.2 mg.% when chewing nothing, 125.5 mg.% when chewing paraffin, 63 mg.%

in fast secreting saliva, and 39 mg.% in slow secreting saliva.[9] Another source states that the concentration of chloride in saliva is 120 mg.% in stimulated saliva.[38] The concentration of chloride increases with the increased secretion of saliva.[8] The calcium and magnesium concentrations in human saliva are 3.0-8.0 meq./liter and 0.16-1.06 meq./liter respectively.[7]

With the awareness that chloride is present in saliva, it has been shown that chloride is actually necessary for the activation of the salivary amylase.[6] Salt free salivary amylase is not active. In dialyzed saliva a precipitate appears simultaneously with the loss of amylase activity. (The formation of such a precipitate was not observed by this author in this present study.) On the addition of salt, the precipitate dissolves and the enzyme activity returns. Of the various salts, the greatest solubility of the amylase and its maximum activity are obtained with 0.05 M sodium chloride.[32]

Sodium chloride up to 0.01% increases the activity of human salivary amylase. At higher concentrations the activity remains constant.[35] At 1.07% NaCl, the temperature optimum goes from 28° to 43° C.[35]

Hydrolytic activity of amylase is at a maximum between 0.034-1.4% NaCl.[13]

Human salivary amylase loses 15% of its activity when dialyzed for several days at 2° C. against an aqueous

solution containing NH_4OH, but this inactivity was reversed upon the addition of 0.01 M NaCl.[27,28,29]

No apparent loss of enzyme activity can be seen over a twelve hour period at a pH of 4.5-11.0 but a decrease can be seen after twelve hours.[27,28,29]

This activation of the salivary amylase by the chloride ion seems to be due to binding of the anions. This was shown by electromotive force measurement and conductivity determinations.[34]

There are many opinions concerning the number of amylases contained in saliva. One report showed the separation of three amylases.[21] Another showed two or more zones of amylotytic activity with agar gel electrophoresis.[33] Still others show one zone[1] and another showed four zones of activity.[30]

Chlorides, bromides, iodides of lithium, sodium, potassium, ammonium, magnesium, calcium, and barium hasten hydrolysis of starch by salivary and pancreatic amylases.[10] Sodium, potassium, and ammonium fluorides do not hasten amylolytic action, and at high concentrations the two latter salts actually inhibit amylase activity.[10]

As an example of how critical it is to control variables in an experiment such as this, one author reports that temperature is so important in establishing amylolytic activity

that studies cannot be compared if they were not carried out at the same temperature.[37]

Hanhila investigated the effects of sodium and potassium on amylase activity and concluded the following:

a) K^+ caused greater reaction at low concentrations than Na^+ for both human salivary and swine pancreatic amylases.

b) High concentrations of both K^+ and Na^+ cause a decreased response in human salivary amylase, but not in swine pancreatic amylase.

c) Swine pancreatic amylolytic activity increases at a much slower rate than human salivary amylolytic activity.

The point was that two very similar salts do not produce identical increase in amylolytic activity.[14] Note that Hanhila compared human salivary amylase with swine pancreatic amylase.

It has been shown that the removal of calcium from human salivary amylase inactivates the molecule of amylase. Reactivation is accomplished by the addition of calcium.[42,43]

CHAPTER III

METHODS AND MATERIALS

The effect of potassium, sodium, calcium, and magnesium concentrations on human salivary alpha-amylase was determined. A study of pancreatic amylase was also attempted, but due to unknown reasons none of the numerous samples studied retained amylase activity.

The method used for amylase assay is a modification of the method of Bernfeld.[3] The method differs from that of Bernfeld as follows: (1) Incubation was performed at room temperature instead of 20° C. as matter of convenience. (2) The color reagent contained 1 mg./ml. of 3,5 dinitrosalicylic acid instead of 10 mg./ml.[41] (3) Samples for spectrophotometric observations were diluted 1:8 instead of 1:5 to reduce the total amount of light energy absorbed. (4) The 1% soluble starch solution contained no sodium phosphate buffer or NaCl because both sodium and chloride are experimental parameters.

The dinitrosalicylic acid reaction appears well suited for amylase assay, since the amount of color developed is proportional to the actual number of reducing groups generated. Thus, cleavage of each glucosidic bond will yield

the same increase in color intensity, irrespective of the molecular size of the products formed. Protein does not interfere with the dinitrosalicylic acid reaction.

The activity of amylase diminishes with time, becoming less effective with time. Salivary enzyme samples were discarded 72 hours after collection. Therefore, what was looked for in the experimentation was not a daily duplication of enzymic action, but rather a trend relating enzymic activity to salt concentrations, and a comparison of how the values progressed or declined with respect to each other.

It has been noted that the chloride anion is required to activate amylase after dialysis. For this reason it was decided to utilize the salts, NaCl, KCl, $CaCl_2$, and $MgCl_2$ to be variables in the procedure. In this manner the chloride was present for enzyme activation and the various cations were the experimental variables.

Five concentrations of NaCl, KCl, $CaCl_2$, and $MgCl_2$; 0.0003 M, 0.003 M, 0.03 M, 0.3 M, and 3.0 M, were prepared as follows:

All salts, except $MgCl_2$, were dried in a drying oven prior to weighing to assure a true dry weight. The following weights of each salt were obtained:

$NaCl$ = 17.535 grams
KCl = 22.368 grams
$CaCl_2$ = 17.343 grams
$MgCl_2$ = 30.498 grams

Each salt sample was then quantitatively transferred to a one hundred milliliter volumetric flask and diluted to one hundred milliliters with de-ionized distilled water. A 3 M solution in regards to the chloride ion resulted in all four samples. Aliquots of this 3 M solution were used to make the other four concentrations in the following manner:

10 milliliters of 3.0 M diluted to 100 ml. = 0.3 M
1 milliliter of 3.0 M diluted to 100 ml. = 0.03 M
1 milliliter of 0.3 M diluted to 100 ml. = 0.003 M
1 milliliter of 0.03 M diluted to 100 ml. = 0.0003 M

To prepare the enzyme solutions samples of both human saliva and human pancreatic juice were collected. (The pancreatic juice was provided by Hines V. A. Hospital, Hines, Illinois and was received in frozen form.) The samples were centrifuged for 20 minutes. The supernatant was then dialyzed for 72 hours at 4° C. against four changes of de-ionized H_2O distilled from a Corning Ag-1b still. Before use, the dialysis bags were soaked in the above mentioned water. The enzyme dialyzate was then diluted with de-ionized water 1:500.

The color reagent was prepared by dissolving one hundred milligrams of 3,5 dinitrosalicylic acid in 20 ml. of 2 N NaOH and 50 ml. water; then 30 gm. of sodium-potassium

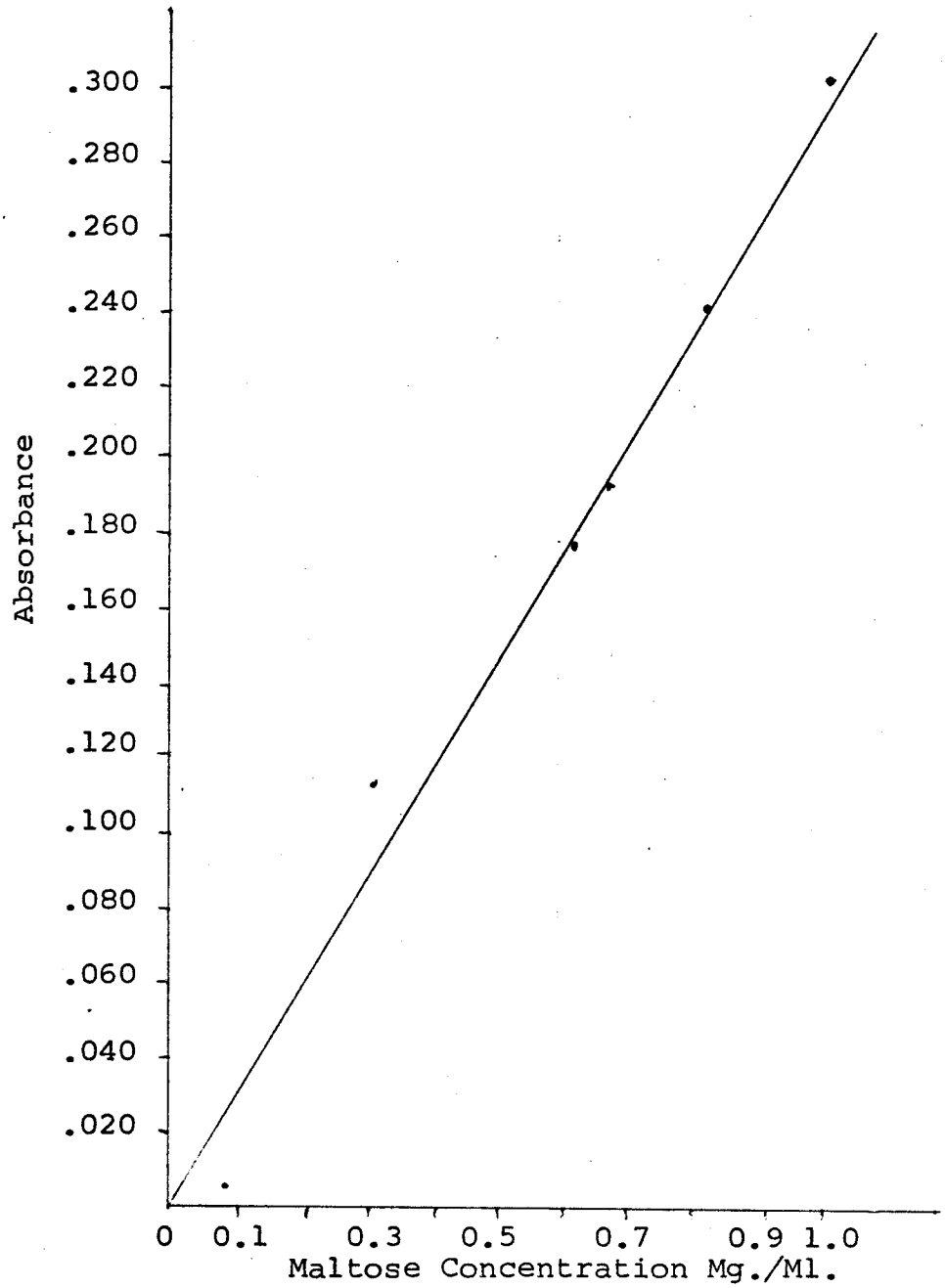
tartrate (Rochelle Salt) was added and the solution was diluted to 100 ml. with water.

The 1% starch solution was prepared as follows: 1 gram of soluble starch was mixed with 2 ml. of distilled water. The starch slurry was then added to 50 ml. of boiling distilled water and heated for one minute. The solution was cooled, diluted to 100 ml., and stored in the refrigerator.

A standard curve relating maltose concentration to the color reaction was obtained by reacting 1 ml. samples of aqueous maltose solution in concentrations 0.1 to 1.0 mg./ml., with 2 ml. of 3,5 dinitrosalicylic acid, in duplicate. These were heated for five minutes in a boiling water bath, cooled immediately in running tap water, and 10 ml. of de-ionized, distilled water was added prior to reading at 540 mμ. on the spectrophotometer. The results are summarized in the following standard curve (Figure 1, page 12A) from the data in Table II (Appendix).

The assay for amylase activity was performed in duplicate with the enzyme dissolved in solutions containing various concentrations of NaCl, KCl, $CaCl_2$, and $MgCl_2$.

As a method of checking accuracy and activity of the reagents on a day-to-day basis, tests were run simultaneously on four additional sets of tubes. In the first set of tubes,



STANDARD CURVE FOR MALTOSE

FIGURE 1

1 mg./ml. maltose solution was used to compare the daily results with the standard curve established. The second set of tubes contained 1 ml. of starch, 2 ml. water, and 2 ml. DNSal. This was used as the reagent blank. The third set of tubes contained 3 ml. water and 2 ml. DNSal. This was the color blank. The fourth set of tubes contained 1 ml. enzyme, 1 ml. starch, 1 ml. water, and 2 ml. DNSal. This set of tubes determined the residual activity, if any, of the salivary amylase after dialysis.

All solutions were kept refrigerated and all assays were performed at room temperature.

The basic protocol for the experiment had fourteen duplicate sets of test tubes. Table I (Page 13A) shows the various volumes and combinations used in the experimental protocol.

In the experiment, the enzyme was always added last. This completed the ingredients and started the reaction. The solution was then mixed and allowed to stand for 12 minutes. At the end of 12 minutes, 2 ml. of DNSal color reagent was added to each test tube thus stopping the reaction. A routine was established whereby 1 ml. of enzyme was added every 30 seconds, mixed, and placed back in the rack. With a total of eleven pairs, there was one minute leeway before the addition

TABLE I

VOLUMES AND COMBINATIONS USED IN THE

EXPERIMENTAL PROTOCOL

TUBE	ENZYME	SALT M/3ml	STARCH	MALTOSE	H_2O	DNSal
1	0	0	0	1 ml.	2 ml.	2 ml.
2	0	0	1 ml.	0	2 ml.	2 ml.
3	0	0	0	0	3 ml.	2 ml.
4	1 ml.	0	1 ml.	0	1 ml.	2 ml.
5	1 ml.	0.0003M	1 ml.	0	0	2 ml.
6	1 ml.	0.003M	1 ml.	0	0	2 ml.
7	1 ml.	0.03M	1 ml.	0	0	2 ml.
8	1 ml.	0.3M	1 ml.	0	0	2 ml.
9	1 ml.	3.0M	1 ml.	0	0	2 ml.
10	1 ml.	0.0003M	1 ml.	0	0	2 ml.
11	1 ml.	0.003M	1 ml.	0	0	2 ml.
12	1 ml.	0.03M	1 ml.	0	0	2 ml.
13	1 ml.	0.3M	1 ml.	0	0	2 ml.
14	1 ml.	3.0M	1 ml.	0	0	2 ml.

of DNSal to the initial tube. It will be noted that numbers 1, 2 and 3 required no timing.

All of the above solutions were then placed simultaneously in a boiling water bath at 100° C. for five minutes and then chilled by immediate immersion in cold running tap water. To each tube, 8 ml. of distilled water was added so the absorbance could be read on the spectrophotometer at 540 mμ. The spectrophotometer was zeroed with the reference cuvette using distilled water. The reference was checked periodically during the reading of the experimental tubes. The results are recorded in Table III (Appendix).

As a statistical method of analyzing the data the Student t-Test was used.

CHAPTER IV

RESULTS

The results of all tests run are shown in Table III (Appendix) and Table IV (Page 16A). The results listed are the effects of Na^+, K^+, Mg^{++}, and Ca^{++} on salivary amylase.

In order to establish a trend, and for comparison purposes, the reagent blank (Tubes #2, Table I) was averaged for each day and this average served as zero for that day. Accordingly, this average was subtracted from the average of each pair of values. In this way an average was then determined for each concentration of the two salts. These values are referred to as units of activity.

A statistical analysis of the listed results shows that the Na^+ and K^+ concentrations result in equal activity at a probability of >0.05.

The Mg^{++} shows lowest activity and the Ca^{++} seems to initiate the highest and most significant activity. A precipitate always formed at 1.0 M concentration of $CaCl_2$ and at 0.1 M and 1.0 M concentrations of $MgCl_2$. These tubes were centrifuged and the precipitate discarded before reading. These readings seem to always indicate a great reduction of activity.

To say these readings after centrifugation are accurate, however, cannot be done unequivocally. Future

research and a different assay method might resolve the question of inhibition of activity by Ca^{++} and Mg^{++} chlorides. (It should be pointed out that both Mg^{++} and Ca^{++} concentrations were half that of Na^{+} and K^{+}).

Table IV (Page 16A) shows a summary of the analytical data. Figure 2 (Page 16B) shows a curve of the effects of the salt concentrations on activity of salivary amylase.

From a statistical point of view the only points that showed a significant difference were as follows:

a) The difference in activity of Na^{+} as compared to Ca^{++} at 0.001 M and 0.1 M. ($P<.05$ and $P<.01$)

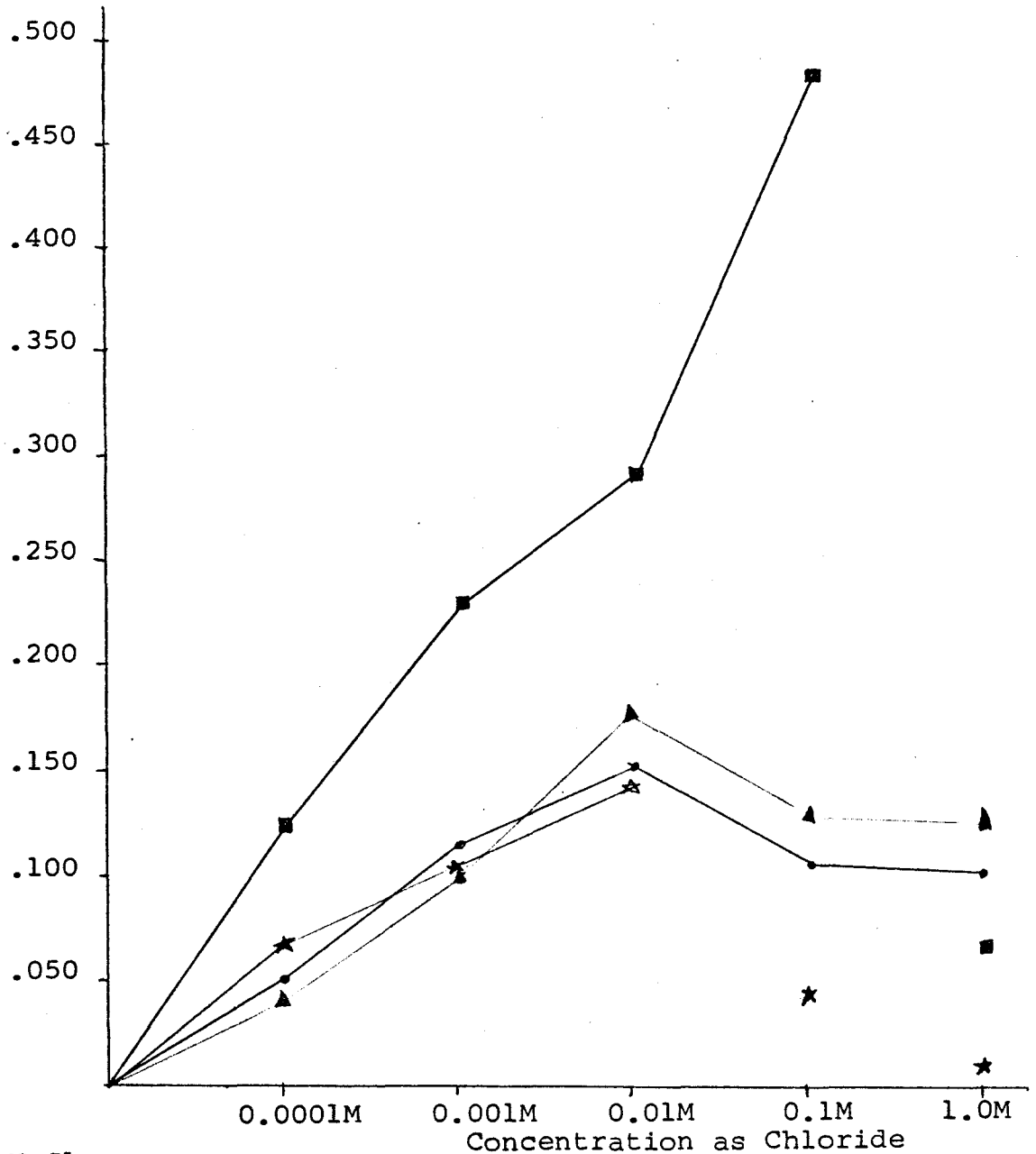
b) The difference in activity of Ca^{++} as compared to Mg^{++} at 0.01 M. ($P<.01$)

TABLE IV

SUMMARY OF ANALYTICAL DATA

TUBE	SALT CONTENT	ABS.	SALT CONTENT	ABS.	SALT CONTENT	ABS.	SALT CONTENT	ABS.
1	---	.205	---	.205	---	.205	---	.205
2	---	0	---	0	---	0	---	0
3	---	0	---	0	---	0	---	0
4	---	.018	---	.018	---	.018	---	.018
5	0.0001M NaCl	.052	0.0001M NaCl	.120	0.0001M NaCl	.073	0.0001M $CaCl_2$	.027
6	0.001	.114	0.001	.168	0.001	.131	0.001	.048
7	0.01	.149	0.01	.241	0.01	.169	0.01	.126
8	0.1	.108	0.1	.192	0.1	.202	0.1	.203
9	1.0	.105	1.0	.184	1.0	.129	1.0	0*
10	0.0001M KCl	.047	0.0001M $CaCl_2$	.126	0.0001M $MgCl_2$	.065	0.0001M $MgCl_2$	.025
11	0.001	.104	0.001	.237	0.001	.115	0.001	.045
12	0.01	.170	0.01	.289	0.01	.145	0.01	.068
13	0.1	.132	0.1	.477	0.1	.043*	0.1	.007*
14	1.0	.130	1.0	.064*	1.0	.011*	1.0	0*

* Precipitate formed



EFFECT OF SALT CONCENTRATION

ON ACTIVITY OF SALIVARY AMYLASE

FIGURE 2

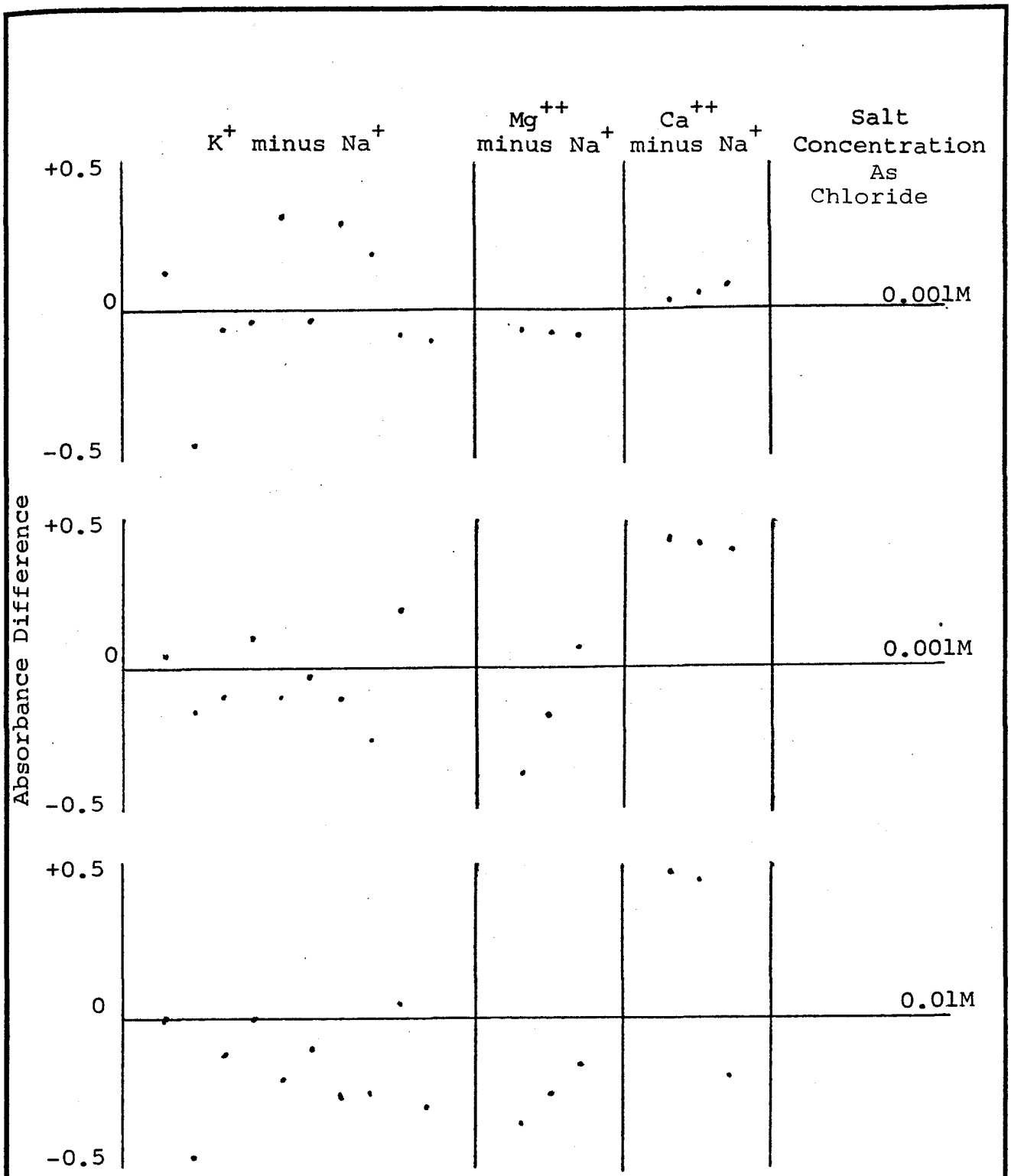
CHAPTER V

DISCUSSION AND CONCLUSIONS

The initial purpose of this study was to investigate the effects of potassium, sodium, magnesium, and calcium ions on salivary and pancreatic amylase. As previously stated, however, I was unable to obtain activity from the enzyme in the pancreatic juice samples. The failure to obtain amylase activity from the pancreatic juice sample, even though it received identical preparation as the salivary sample, leads one to question the conclusion that there is no basic difference between the two enzymes. What this difference is cannot be answered from the results obtained by this research.

Analysis of the analytical data (Figure 3, Page 17A) indicates that potassium chloride gives consistently higher levels of enzyme activity at concentrations of 0.1 M and 1.0 M than does sodium chloride at the same concentrations. At 0.0001 M and 0.001 M the amount of activation is about equal for both salts. Statistic evaluation, however, showed no significant differences between NaCl and KCl at any concentrations. Further studies using larger samples may show some significant difference.

Magnesium chloride activates salivary amylase to a lesser extent when compared to sodium chloride at the same concentrations of chloride. (It should be noted that Mg^{++} is half the concentration of Na^{+}.) The aforementioned occurrance of



SCATTERGRAMS OF ANALYTICAL DATA

FIGURE 3

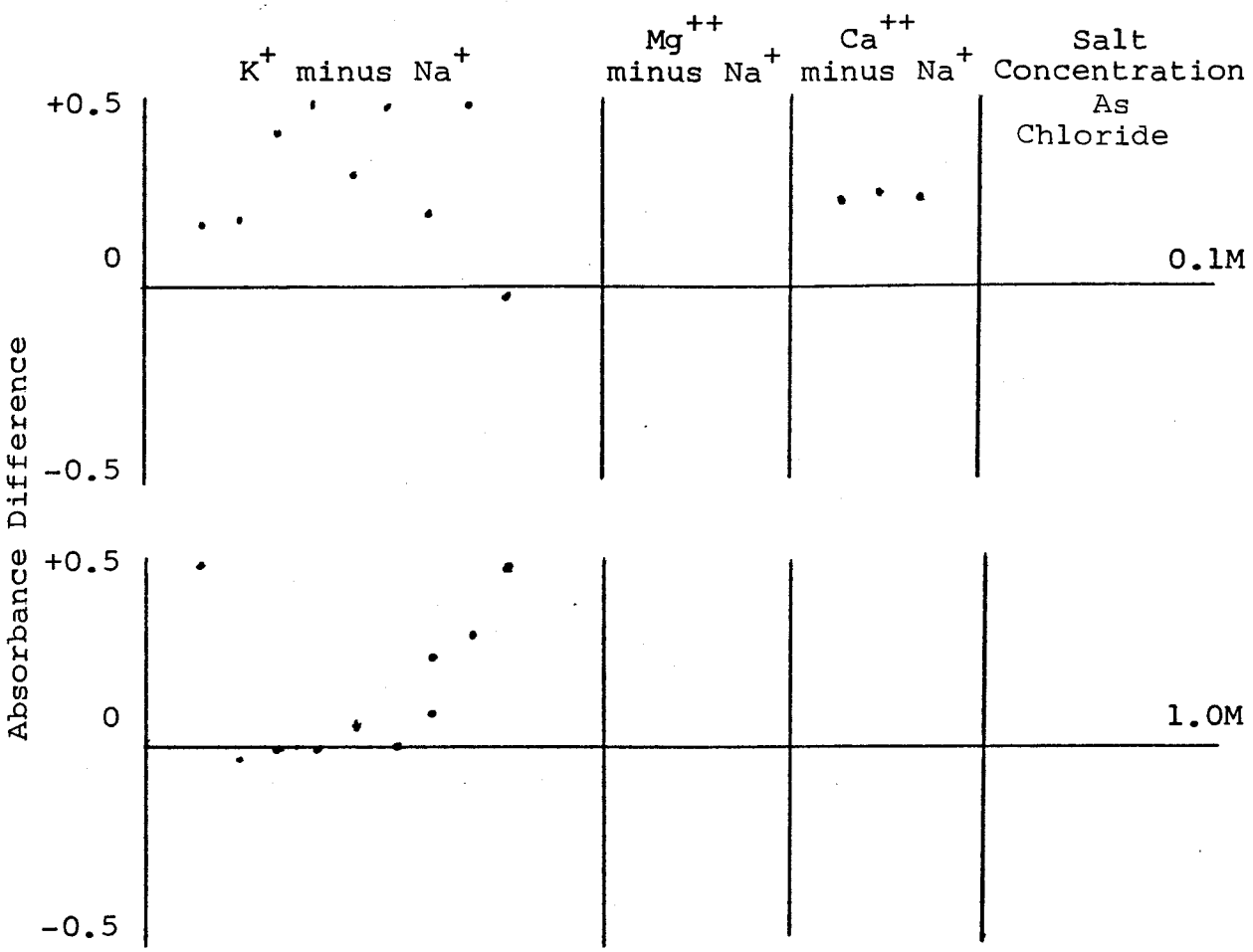


FIGURE 3 (CONTINUED)

precipitates at 0.1 M and 1.0 M concentrations of Mg^{++} (chloride concentration) prevents the formation of a positive conclusion but it would appear from the data that magnesium may in fact be inhibitory. The magnitude of this "inhibition" increases as the concentration increases from 0.001 M to 0.01 M.

The effect of calcium chloride is opposite to that of magnesium. It activates salivary amylase to a much greater degree than does sodium chloride at the same levels of chloride. The extent of this increase in activation is positively related to the concentration. It is least when the concentration is low and greatest at 0.1 M (chloride concentration). The occurrance of a precipitate at 1.0 M does not permit a conclusion about activation at this concentration.

One might be tempted to conclude from the sodium, potassium, and magnesium data that there is little effect by cations on the activity of salivary amylase but the calcium data indicates otherwise. How might this activation by calcium be explained? One possible answer is found in the research of Stein, Hsiu and Fischer mentioned in Chapter II of this study.[42,43] They found if calcium is removed from human salivary amylase it becomes inactive. Activity, however, returns upon the addition of calcium. With these facts in mind one can postulate why the $CaCl_2$ seems to have more effect on amylases

activity than NaCl, KCl, and $MgCl_2$. When the enzyme samples were dialyzed the calcium was possibly removed from the amylase. One can see that the activity of the amylase samples would be more effected by the addition of $CaCl_2$ than NaCl, KCl and $MgCl_2$ if dialyses had removed the calcium and the presence of calcium is necessary for optimal activity. Earlier reports, however, indicated that the removal of calcium could be achieved only under relatively drastic conditions which led to irreversible inactivation and, presumably, denaturation of the protein.[42,43] A future study using CaOH for a dialysing solution instead of water might provide more answers on this subject.

As noted previously, solutions containing 0.1 M and 1.0 M $MgCl_2$, and 1.0 M $CaCl_2$ were found to form a precipitate when DNSal reagent was added to the enzyme-substrate mixture. This precipitate prevented me from obtaining reliable absorbance readings. Readings taken after removal of the precipitate by centrifugation would seem to indicate a reduction in activity. This reduction, however, cannot be verified until an assay is utilized that does not cause a precipitate at the above mentioned concentrations. The reason for this precipitate formation and how to prevent its formation was beyond the scope of this study.

From the nature and extent of the present research it is not possible to determine a mechanism which accounts for the observed effects but the data observed here is in agreement with previous statements in the literature concerning calcium. The stabilizing effect of calcium salts on the activity of alpha-amylase was first noted by Wallerstein sixty years ago when he patented their use in connection with the brewing process.[44] In the course of their studies on the comparative structures of alpha-amylases Stein and Fischer noted that the enzymes were maximally resistant towards proteases when combined with divalent metal ions.[45] They found that alpha-amylases were highly susceptible to proteolysis when these ions are removed so that in the presence of metal binding agents even trace amounts of proteolytic enzymes leads to rapid inactivation. The postulated loss of calcium by the pancreatic amylase during dialysis and in the presence of a fluid especially rich in proteolytic enzymes would easily explain the absence of amylolytic activity after dialysis against water.

Almost forty years ago Ernstrom reported that sodium chloride protected salivary amylase against heat denaturation.[46] Schneyer's study on the effect of sodium and potassium ions on the temperature behavior of salivary amylase presented further evidence for the role of the cation in protection against

denaturation.[47] The results of his study show that "...although the chloride ion is present in equal concentration in each case, protection by potassium is always greater than by sodium ion of the same concentration. It is apparent, therefore, that not only is potassium a better protective agent than sodium, but it is the cation which is the protective factor." Schneyer went on to propose that the observed variation in enzymic activity with salt concentration was related to the relative proportions of the enzyme in the native and denatured states. And that this was related to the previous mentioned effect of the cations. The results from this study showed a trend toward agreement with Schneyer's work.

The nature of this effect in terms of the cations themselves has not been explained. It is unrelated to the crystal radii of the ions which are in the following order: K^+ Ca^{++} Na^+ Mg^{++} [48] or to the electronegativity of values which are as follows: Mg^{++} Ca^{++} Na^+ K^+ [49].

The results of this study have opened the door for many future studies. There may be variations of the cation, i.e., NaCl vs. NH_4Cl, LiCl, etc. The buffer could be a variable by eliminating the buffer as in this study, or by using buffers such as phosphates, Tris, and citrates. The pH could be varied to study the pH effect on activity. The substrate could be varied using other starches, looking for

inhibitor characteristics as well as substrate affinity. Are the disaccharides equally effective as inhibitors using these salt systems? The anions could be varied using the other three halogens, Br^-, I^-, and F^-, with the various cations. The enzyme source could also be varied using serum and urinary amylase. The activity of a human pancreatic amylase could possibly be obtained so that it could be compared with human salivary amylase. The utilization of another assay to investigate the effects of higher concentrations of $CaCl_2$ and $MgCl_2$ on salivary amylase without the interference of precipitate formation. Such an assay could be preformed by serial testing of enzyme starch solutions with iodine and measuring the time required to arrive at no color reaction.

CHAPTER VI

SUMMARY

Some relationships were established on the basis of each individual test. The following summary seemed to be justified on the basis of the results of this study:

1) $CaCl_2$ caused the greatest increase in activity of the salivary amylase.
2) $MgCl_2$ caused the least activity of the salivary amylase.
3) NaCl and KCl caused similar activity of the salivary amylase.
4) High concentrations of both $CaCl_2$ and $MgCl_2$ caused a precipitate to form.
5) There is no apparent reason for the difference in the results reported here and by Hanhila. This disparity will be the subject of further research.

6) The data are consistant with the observations of Wallerstein[44], Ernstrom[46], and Schneyer[47] with regard to the relative efficacy of sodium, potassium and calcium ions in stabilizing alpha-amylase.

7) The effect of these ions upon the activity of alpha-amylase does not appear to be related to their crystal radii or their electro-negativity.

APPENDIX

TABLE II

STANDARD CURVE DATA FOR MALTOSE

TUBE	MALTOSE CONTENT	A	B	C	AVERAGE	AVERAGE -BLANK
1	1 mg./ml.	.320	.320	.320	.320	.300
2	0.8 mg./ml.	.258	.260	.261	.260	.240
3	0.66 mg./ml.	.215	.210	.210	.212	.192
4	0.60 mg./ml.	.189	.188	.185	.187	.167
5	0.30 mg./ml.	.128	.128	.129	.128	.108
6	0.098 mg./ml.	.026	.025	.025	.025	.005
7	Blank	.021	.020	.019	.020	0

TABLE III

ACTUAL ANALYTICAL DATA

2-11-71 (V.M.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.260	.261	.261	.230
2	0	.031	.030	.031	0
3	0	.021	.020	.021	0
4	0	.031	.031	.031	0
5	0.0003 NaCl	.082	.079	.081	.050
6	0.003	.121	.120	.121	.090
7	0.03	.143	.140	.142	.111
8	0.3	.128	.130	.129	.098
9	3.0	.092	.100	.096	.065
10	0.0003 KCl	.075	.065	.071	.040
11	0.003	.101	.095	.098	.067
12	0.03	.101	.102	.102	.071
13	0.3	.099	.108	.104	.073
14	3.0	.121	.111	.116	.085

Molarity of salt solution added

TABLE III (CONTINUED)

2-20-71 (W.R.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.250	.245	.248	.206
2	0	.041	.042	.042	0
3	0	.020	.019	.020	0
4	0	.070	.068	.069	.027
5	0.0003 NaCl	.129	.130	.130	.088
6	0.003	.155	.155	.155	.113
7	0.03	.182	.175	.179	.137
8	0.3	.162	.135	.149	.107
9	3.0	.159	.157	.158	.116
10	0.0003 KCl	.055	.081	.068	.026
11	0.003	.151	.141	.146	.104
12	0.03	.105	.088	.096	.054
13	0.3	.160	.162	.161	.119
14	3.0	.151	.161	.156	.114

Molarity of salt solution added

TABLE III (CONTINUED)

2-21-71 (W.R.)

TUBE	SALT CONTENT#	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.260	.260	.260	.194
2	0	.063	.068	.066	0
3	0	.021	.020	.021	0
4	0	.059	.058	.059	0
5	0.0003 NaCl	.060	.055	.058	0
6	0.003	.100	.110	.105	.039
7	0.03	.120	.121	.121	.055
8	0.3	.105	.110	.108	.042
9	3.0	.121	.110	.116	.050
10	0.0003 KCl	.078	.080	.079	.013
11	0.003	.110	.108	.109	.043
12	0.03	.120	.120	.120	.054
13	0.3	.120	.125	.123	.057
14	3.0	.255	.235	.245	.179

Molarity of salt solution added

TABLE III (CONTINUED)

2-27-71(F.S.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.252	.250	.251	.202
2	0	.055	.043	.049	0
3	0	.037	.037	.037	0
4	0	.045	.043	.044	0
5	0.0003 NaCl	.145	.148	.047	0
6	0.003	.200	.180	.190	.141
7	0.03	.200	.210	.205	.156
8	0.3	.165	.145	.155	.106
9	3.0	.120	.140	.130	.081
10	0.0003 KCl	.065	.071	.068	.019
11	0.003	.160	.170	.165	.116
12	0.03	.165	.180	.173	.124
13	0.3	.230	.200	.215	.166
14	3.0	.142	.179	.161	.112

Molarity of salt solution added

TABLE III (CONTINUED)

3-6-71 (F.S.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.250	.245	.248	.208
2	0	.040	.039	.040	0
3	0	.031	.032	.032	0
4	0	.040	.038	.039	0
5	0.0003 NaCl	.112	.114	.113	.073
6	0.003	.155	.143	.149	.109
7	0.03	.190	.180	.185	.145
8	0.3	.170	.165	.168	.128
9	3.0	.150	.160	.155	.115
10	0.0003 KCl	.105	.105	.105	.065
11	0.003	.170	.170	.170	.130
12	0.03	.202	.180	.191	.151
13	0.3	.160	.170	.165	.125
14	3.0	.210	.240	.225	.185

Molarity of salt solution added

TABLE III (CONTINUED)

3-13-71 (F.S.)

TUBE	SALT CONTENT#	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.230	.225	.227	.187
2	0	.040	.040	.040	0
3	0	.032	.033	.033	0
4	0	.050	.049	.050	.010
5	0.0003 NaCl	.129	.135	.132	.092
6	0.003	.200	.200	.200	.160
7	0.03	.239	.255	.247	.207
8	0.3	.185	.195	.190	.150
9	3.0	.190	.192	.191	.151
10	0.0003 KCl	.122	.120	.121	.081
11	0.003	.188	.190	.189	.149
12	0.03	.230	.240	.235	.195
13	0.3	.235	.230	.233	.193
14	3.0	.190	.192	.191	.151

Molarity of salt solution added

TABLE III (CONTINUED)

3-13-71 (W.R.)

TUBE	SALT CONTENT#	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.229	.225	.227	.188
2	0	.038	.039	.039	0
3	0	.030	.032	.031	0
4	0	.045	.049	.047	.008
5	0.0003 NaCl	.125	.129	.127	.088
6	0.003	.181	.179	.180	.141
7	0.03	.219	.224	.222	.183
8	0.3	.179	.175	.177	.098
9	3.0	.185	.190	.187	.108
10	0.0003 KCl	.120	.125	.123	.044
11	0.003	.185	.190	.187	.108
12	0.03	.220	.225	.223	.144
13	0.3	.229	.225	.227	.148
14	3.0	.189	.190	.190	.111

Molarity of salt solution added

TABLE III (CONTINUED)

3-20-71 (W.R.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.260	.259	.260	.223
2	0	.035	.038	.037	0
3	0	.031	.030	.031	0
4	0	.045	.043	.044	.007
5	0.0003 NaCl	.055	.049	.052	.015
6	0.003	.140	.135	.138	.101
7	0.03	.190	.180	.185	.148
8	0.3	.145	.135	.140	.103
9	3.0	.150	.145	.148	.111
10	0.0003 KCl	.082	.082	.082	.045
11	0.003	.130	.120	.125	.088
12	0.03	.161	.159	.160	.123
13	0.3	.155	.165	.160	.123
14	3.0	.140	.150	.145	.108

Molarity of salt solution added

TABLE III (CONTINUED)

3-21-71 (W.R.)

TUBE	SALT CONTENT#	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.261	.255	.258	.221
2	0	.035	.039	.037	0
3	0	.030	.025	.028	0
4	0	.061	.055	.058	.021
5	0.0003 NaCl	.125	.130	.128	.091
6	0.003	.190	.181	.186	.149
7	0.03	.230	.250	.240	.203
8	0.3	.180	.190	.185	.148
9	3.0	.185	.182	.184	.147
10	0.0003 KCl	.120	.128	.124	.087
11	0.003	.182	.185	.184	.147
12	0.03	.225	.235	.230	.193
13	0.3	.230	.229	.230	.193
14	3.0	.182	.185	.184	.147

Molarity of salt solution added

TABLE III (CONTINUED)

3-21-71(W.R.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.251	.250	.251	.209
2	0	.040	.043	.042	0
3	0	.035	.035	.035	0
4	0	.050	.049	.050	.008
5	0.0003 NaCl	.060	.059	.060	.018
6	0.003	.145	.140	.143	.101
7	0.03	.195	.183	.189	.147
8	0.3	.150	.141	.146	.104
9	3.0	.153	.149	.151	.109
10	0.0003 KCl	.089	.090	.090	.048
11	0.003	.135	.128	.132	.090
12	0.03	.165	.163	.164	.122
13	0.3	.161	.170	.166	.124
14	3.0	.145	.159	.152	.110

Molarity of salt solution added

TABLE III (CONTINUED)

3-12-71 (W.R.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.260	.258	.259	.215
2	0	.045	.043	.044	0
3	0	.036	.036	.036	0
4	0	.065	.055	.060	.016
5	0.0003 NaCl	.158	.148	.153	.109
6	0.003	.182	.190	.188	.144
7	0.03	.275	.270	.273	.229
8	0.3	.235	.237	.236	.192
9	3.0	.235	.240	.237	.193
10	0.0003 $CaCl_2$	.160	.150	.155	.111
11	0.003	.230	.230	.230	.186
12	0.03	.330	.322	.326	.282
13	0.3	.560	.460	.510	.466
14	3.0	.099*	.085*	.092	.048

* Precipitate formed
# Molarity of salt solution added

TABLE III (CONTINUED)

3-14-71 (W.R.)

TUBE	SALT CONTENT#	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.255	.251	.253	.203
2	0	.050	.049	.050	0
3	0	.034	.034	.034	0
4	0	.225	.229	.227	.177
5	0.0003 NaCl	.142	.145	.143	.093
6	0.003	.185	.190	.187	.137
7	0.03	.250	.245	.247	.197
8	0.3	.280	.279	.280	.230
9	3.0	.180	.181	.181	.131
10	0.0003 $MgCl_2$	.130	.140	.135	.085
11	0.003	.195	.190	.193	.143
12	0.03	.230	.245	.238	.188
13	0.3	.115*	.100*	.108	.058
14	3.0	.080*	.070*	.075	.025

* Precipitate formed
# Molarity of salt solution added

TABLE III (CONTINUED)

4-4-71 (G.S.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.230	.220	.225	.209
2	0	.015	.017	.016	0
3	0	.013	.010	.012	0
4	0	.059	.059	.059	.043
5	0.0003 NaCl	.151	.149	.150	.134
6	0.003	.200	.215	.208	.192
7	0.03	.270	.269	.270	.254
8	0.3	.205	.210	.208	.192
9	3.0	.189	.190	.190	.174
10	0.0003 $CaCl_2$	.151	.160	.156	.140
11	0.003	.241	.235	.238	.222
12	0.03	.380	.375	.378	.362
13	0.3	.500	.490	.495	.479
14	3.0	.100*	.090*	.095	.079

*Precipitate formed

Molarity of salt solution added

TABLE III (CONTINUED)

4-4-71(G.S.)

TUBE	SALT CONTENT#	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.165	.161	.163	.143
2	0	.016	.019	.018	0
3	0	.012	.011	.012	0
4	0	.040	.030	.035	.017
5	0.0003 NaCl	.065	.075	.070	.052
6	0.003	.140	.143	.142	.124
7	0.03	.155	.160	.158	.140
8	0.3	.191	.192	.192	.174
9	3.0	.139	.149	.144	.126
10	0.0003 $MgCl_2$	.065	.061	.063	.045
11	0.003	.100	.111	.106	.088
12	0.03	.121	.121	.121	.103
13	0.3	.040*	.049*	.045	.027
14	3.0	.010*	.010*	.010	0

* Precipitate formed
# Molarity of salt solution added

TABLE III (CONTINUED)

4-10-71 (J.D.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.245	.239	.242	.212
2	0	.030	.030	.030	0
3	0	.025	.023	.024	0
4	0	.061	.059	.061	.031
5	0.0003 NaCl	.155	.149	.147	.117
6	0.003	.191	.203	.197	.167
7	0.03	.273	.270	.272	.242
8	0.3	.220	.225	.223	.193
9	3.0	.212	.215	.214	.184
10	0.0003 $CaCl_2$	.156	.155	.156	.126
11	0.003	.235	.233	.234	.204
12	0.03	.255	.249	.252	.222
13	0.3	.530	.500	.515	.485
14	3.0	.100*	.089*	.095	.065

* Precipitate formed

Molarity of salt solution added

TABLE III (CONTINUED)

4-10-71 (J.D.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.210	.205	.208	.174
2	0	.033	.035	.034	0
3	0	.023	.023	.023	0
4	0	.050	.045	.048	.014
5	0.0003 NaCl	.103	.110	.107	.073
6	0.003	.163	.167	.165	.131
7	0.03	.203	.203	.203	.169
8	0.3	.235	.235	.235	.201
9	3.0	.160	.165	.163	.129
10	0.0003 $MgCl_2$	.099	.100	.100	.066
11	0.003	.145	.150	.148	.114
12	0.03	.175	.183	.179	.145
13	0.3	.079*	.075*	.077	.043
14	3.0	.045*	.040*	.043	.009

* Precipitate formed

Molarity of salt solution added

TABLE III (CONTINUED)

3-20-71(W.R.)

TUBE	SALT CONTENT#	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.250	.240	.245	.206
2	0	.039	.038	.039	0
3	0	.031	.030	.031	0
4	0	.038	.036	.037	0
5	0.0003 $CaCl_2$	.059	.049	.054	.015
6	0.003	.070	.080	.075	.036
7	0.03	.149	.155	.152	.113
8	0.3	.219	.220	.220	.181
9	3.0	.032*	.035*	.034	0
10	0.0003 $MgCl_2$	.060	.050	.055	.016
11	0.003	.090	.080	.085	.046
12	0.03	.095	.090	.093	.054
13	0.3	.045**	.045**	.045	.006
14	3.0	.032*	.030*	.031	0

* Precipitate formed
** Red Precipitate formed
# Molarity of salt solution added

TABLE III (CONTINUED)

3-20-71 (W.R.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.259	.255	.257	.220
2	0	.037	.037	.037	0
3	0	.030	.030	.030	0
4	0	.039	.035	.037	0
5	0.0003 $CaCl_2$	.078	.068	.073	.036
6	0.003	.100	.095	.098	.061
7	0.03	.185	.175	.180	.143
8	0.3	.245	.255	.250	.213
9	3.0	.032*	.030*	.031	0
10	0.0003 $MgCl_2$	.075	.060	.068	.031
11	0.003	.081	.075	.078	.041
12	0.03	.120	.112	.116	.079
13	0.3	.051**	.052**	.052	.015
14	3.0	.031*	.035*	.033	0

* Precipitate formed
** Red precipitate formed
# Molarity of salt solution added

TABLE III (CONTINUED)

3-21-71 (W.R.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.255	.248	.252	.214
2	0	.038	.038	.038	0
3	0	.031	.030	.031	0
4	0	.039	.036	.038	0
5	0.0003 $CaCl_2$	.069	.059	.064	.026
6	0.003	.085	.088	.087	.049
7	0.03	.167	.165	.166	.128
8	0.3	.232	.238	.235	.197
9	3.0	.032*	.033*	.033	0
10	0.0003 $MgCl_2$	.068	.055	.062	.024
11	0.003	.086	.078	.082	.044
12	0.03	.108	.101	.105	.067
13	0.3	.048**	.049**	.049	.011
14	3.0	.032*	.033*	.033	0

* Precipitate formed
** Red precipitate formed

Molarity of salt solution added

TABLE III (CONTINUED)

3-28-71 (W.R.)

TUBE	SALT CONTENT #	A	B	AVERAGE A & B	AVERAGE-BLANK
1	0	.250	.249	.250	.206
2	0	.045	.043	.044	0
3	0	.039	.035	.037	0
4	0	.049	.045	.047	.003
5	0.0003 $CaCl_2$	.075	.079	.077	.033
6	0.003	.090	.089	.090	.046
7	0.03	.181	.189	.185	.141
8	0.3	.261	.269	.265	.221
9	3.0	.041*	.045*	.043	0
10	0.0003 $MgCl_2$	.069	.079	.074	.030
11	0.003	.098	.091	.095	.051
12	0.03	.121	.115	.118	.074
13	0.3	.059*	.050*	.055	.011
14	3.0	.040*	.040*	.040	0

* Precipitate formed
# Molarity of salt solution added

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APPROVAL SHEET

The thesis submitted by William MacDonald Reeves has been read and approved by the faculty of the Department of Oral Biology of Loyola University.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

21 May 1971
Date

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